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(54) Human MYT-1 kinase clone

(57) Human Myt-1 kinase polypeptides and DNA (RNA) encoding such enzyme and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such human Myt-1 kinase in the development of treatments for cancers such as leukemias, solid tumors and metastases, chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis, proliferative vascular diseases such as restenosis, proliferative ocular disorders such as diabetic retinopathy and macular degeneration, and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas, among others, are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides.

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Description

Field of the Invention

This invention relates, in part, to newly identified polynucleotides and polypeptides, variants and derivatives of the polynucleotides and polypeptides, processes for making the polynucleotides and the polypeptides, and their variants and derivatives, agonists and antagonists of the polypeptides, and uses of the polynucleotides, polypeptides, variants, derivatives, agonists and antagonists. In particular, in these and in other regards, the invention relates to novel polynucleotides and polypeptides of the family of Cdc-regulatory kinases, hereinafter referred to as human Myt-1 kinase.

Background of the Invention

Cyclin dependent kinases (CDKs) are a family of serine/threonine kinases that are essential to cell cycle progression. Consequently, the activities of these kinases are tightly regulated. In mammals, at least seven different CDKs have been described to date and have been characterized as CDKs 1-7. They are well conserved, sharing 40 to 75% identity. In addition, extensive similarity has been shown with other serine/threonine protein kinases within their catalytic domains. See Fines, J., *Semin. Cell Biol.* 1994, *5*, 399-408; Morgan, D.O., *Nature*, 1995, *374*, 131-134; and Nigg, E.A., *Bioessays*, 1995, *17*, 471-480. Various mechanisms to regulate CDK activity are used to ensure that the cell's normal cycle is tightly controlled and yet remains exquisitely sensitive to changes in the environment. Lees, E., *Curr. Opin. Cell Biol.*, 1995, *7*, 773-780.

For example, entry of cells into mitosis is initiated by the M phase-promoting factor (MPF), a complex of the Cdc2 protein kinase and cyclin B. Proper regulation of MPF ensures that mitosis occurs only after earlier phases of the cell cycle are complete. Phosphorylation of Cdc2 at Tyr^{15} and Thr^{14} suppresses the activity of MPF during interphase. At G_2-M transition, the Cdc2 is dephosphorylated at Tyr^{15} and Thr^{14}, allowing MPF to phosphorylate its mitotic substrates.

A distinct family of Cdc-regulatory kinases, referred to as Wee-1, has been identified and characterized. Wee-1 was first identified in the fission yeast *Schizosaccharomyces pombe* as an important negative regulator of mitosis. Russell, P. and Nurse, P., *Cell*, 1987, *49*, 559. Homologs of Wee-1 have since been identified in at least six other organisms, in human and *Xenopus*. WEE-1 is a soluble enzyme that phosphorylates Cdc2 on Tyr^{15}, but not on Thr^{14}. Mueller *et al.*, *Mol. Biol. Cell*, 1995, *6*, 119; McGowan, C.H. and Russell, P., *EMBO J.*, 1993, *12*, 75; Parker, L.L. and Piwnica-Worms, H., *Science*, 1992, *257*, 1955; and McGowan, C.H. and Russell, P., *EMBO J.*, 1995, *14*, 2166; Watnabe, *et al.*, *ibid*, p. 1878.

A Thr^{14}-specific kinase activity has been detected in the membrane fraction of *Xenopus* egg extracts. Atherton-Fessler, *et al.*, *Mol. Cell. Biol.*, 1994, *5*, 989; Kornbluth, *et al.*, *ibid.*, p. 273. It has also been demonstrated using extracts of *Xenopus* eggs that this Thr^{14}-specific kinase is tightly membrane associated. Kornbluth *et al.*, *Mol. Biol. Cell*, 1994, *5*, 273-282. Further, the Thr^{14}-specific Cdc kinase, referred to as *Xenopus* Myt-1 membrane-associated inhibitory kinase, was recently shown to be an important regulator of Cdc2/cyclin B kinase activity. Mueller *et al.*, *Science*, 1995, *270*, 86-90. Conceptual translation of the *Xenopus* gene encoding Myt-1 revealed that it is most similar to the Wee-1 family of kinases. Thus, Myt-1 is a subclass of the Wee-1 family.

Regulation of Myt-1 kinase offers a means of controlling a critical event in the cell cycle. Inhibition of Myt-1 kinase activity is believed to result in de-regulation of the timing for entry of cells into mitosis. This generally results in catastrophic mitosis and cell death due to the cells entry into mitosis before all essential proteins and/or DNA is produced. Thus, it is believed that inhibition of Myt-1 activity has utility in treating cancers, such as leukemias, solid tumors and metastases; chronic inflammatory proliferative diseases, such as psoriasis and rheumatoid arthritis; proliferative cardiovascular diseases, such as restenosis; proliferative ocular disorders, such as diabetic retinopathy and macular degeneration; and benign hyperproliferative diseases, such as benign prostatic hypertrophy and hemangiomas.

Clearly there is a need for identification and characterization of human homologs of Myt-1 kinase.

Summary of the Invention

Toward these ends, and others, it is an object of the present invention to provide polypeptides, *inter alia*, that have been identified as novel human Myt-1 kinase by homology between the amino acid sequence set out in Figure and known amino acid sequences of other proteins such as *Xenopus* Myt-1 kinase.

It is a further object of the invention, moreover, to provide polynucleotides that encode human Myt-1 kinase, particularly polynucleotides that encode the polypeptide herein designated by SEQ ID NO:2.

In a particularly preferred embodiment of this aspect of the invention, the polynucleotide comprises the region encoding human Myt-1 kinase in the sequence set out in Figure 1.

In accordance with this aspect of the invention, there are provided isolated nucleic acid molecules encoding human Myt-1 kinase, including mRNAs, cDNAs, genomic DNAs and fragments and, in further embodiments of this aspect of

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the invention biologically diagnostically clinically or therapeutically useful variants analogs or derivatives thereof including fragments of the variants analogs and derivatives

Among the particularly preferred embodiments of this aspect of the invention are naturally occurring allelic variants of human Myt-1 kinase

It also is an object of the invention to provide Myt-1 kinase polypeptides particularly human Myt-1 kinase polypeptides that may be employed for therapeutic purposes for example in the treatment of cancers such as leukemias solid tumors and metastases chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis proliferative cardiovascular diseases such as restenosis proliferative ocular disorders such as diabetic retinopathy and macular degeneration and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas among others

In accordance with this aspect of the invention there are provided novel polypeptides of human origin referred to herein as human Myt-1 kinase as well as biologically diagnostically or therapeutically useful fragments variants and derivatives thereof variants and derivatives of the fragments and analogs of the foregoing

Among the particularly preferred embodiments of this aspect of the invention are variants of human Myt-1 kinase encoded by naturally occurring alleles of the human Myt-1 kinase gene

In accordance with another aspect of the present invention there are provided methods of screening for compounds which bind to and activate or inhibit activation of the kinase of the present invention

It is another object of the invention to provide a process for producing the aforementioned polypeptides polypeptide fragments variants and derivatives fragments of the variants and derivatives and analogs of the foregoing In a preferred embodiment of this aspect of the invention there are provided methods for producing the aforementioned human Myt-1 kinase polypeptides comprising culturing host cells having expressibly incorporated therein an exogenously-derived human Myt-1 kinase-encoding polynucleotide under conditions for expression of human Myt-1 kinase in the host expressing the human Myt-1 kinase in the host cells and then recovering the expressed polypeptide from the host cells

In accordance with another object of the invention there are provided products compositions processes and methods that utilize the aforementioned polypeptides and polynucleotides for research biological clinical and therapeutic purposes *inter alia*

In accordance with certain preferred embodiments of this aspect of the invention there are provided products compositions and methods *inter alia* for among other things assessing human Myt-1 kinase expression in cells by determining human Myt-1 kinase polypeptides or human Myt-1 kinase-encoding mRNA to treat cancers such as leukemias solid tumors and metastases chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis proliferative cardiovascular diseases such as restenosis proliferative ocular disorders such as diabetic retinopathy and macular degeneration and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas among others *in vitro*, *ex vivo* or *in vivo* by exposing cells to human Myt-1 kinase polypeptides or polynucleotides as disclosed herein assaying genetic variation and aberrations such as defects in human Myt-1 kinase genes and administering a human Myt-1 kinase polypeptide or polynucleotide to an organism to augment human Myt-1 kinase function or remediate human Myt-1 kinase dysfunction

In accordance with still another embodiment of the present invention there is provided a process of using such activating compounds to stimulate enzyme of the present invention for the treatment of conditions related to the underexpression of human Myt-1 kinase

In accordance with another aspect of the present invention there is provided a process of using such inhibiting compounds for treating conditions associated with over-expression of the human Myt-1 kinase

In accordance with yet another aspect of the present invention there is provided non-naturally occurring synthetic isolated and or recombinant human Myt-1 kinase polypeptides which are fragments consensus fragments and or sequences having inhibitory activity against those of at least one domain of the human Myt-1 kinase of the present invention such polypeptides being capable of modulating quantitatively or qualitatively human Myt-1 kinase binding to its substrate or ligands

In accordance with still another aspect of the present invention there are provided synthetic or recombinant human Myt-1 kinase polypeptides conservative substitution and domain mutants thereof and nucleic acid molecules encoding the aforementioned polypeptides compositions and methods that can be useful as potential modulators of human Myt-1 kinase function by binding to the enzyme or modulating enzyme binding due to their expected biological properties which may be used in diagnostic therapeutic and/or research applications

It is still another object of the present invention to provide synthetic isolated or recombinant polypeptides which are designed to inhibit or mimic various human Myt-1 kinase or fragments thereof

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided probes that hybridize to human Myt-1 kinase sequences

In certain additional preferred embodiments of this aspect of the invention there are provided antibodies against human Myt-1 kinase polypeptides In certain particularly preferred embodiments in this regard the antibodies are highly

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selective for human Myt-1 kinase.

In accordance with another aspect of the present invention, there are provided human Myt-1 kinase agonists. Among preferred agonists are molecules that mimic the human Myt-1 kinase enzyme, that bind to human Myt-1 kinase-binding molecules or receptors, and that elicit or augment human Myt-1 kinase-induced responses. Also among preferred agonists are molecules that interact with human Myt-1 kinase or human Myt-1 kinase polypeptides, or with other modulators of human Myt-1 kinase activities, thereby potentiating or augmenting an effect of human Myt-1 kinase or more than one effect of human Myt-1 kinase.

In accordance with yet another aspect of the present invention, there are provided human Myt-1 kinase antagonists. Among preferred antagonists are those which mimic the human Myt-1 kinase enzyme so as to bind to human Myt-1 kinase receptors or binding molecules but not elicit a human Myt-1 kinase-induced response or more than one human Myt-1 kinase-induced response. Also among preferred antagonists are molecules that bind to or interact with human Myt-1 kinase so as to inhibit an effect of human Myt-1 kinase or more than one effect of human Myt-1 kinase. Preferred antagonists also include compounds that prevent expression of human Myt-1 kinase, such antisense agents.

In a further aspect of the invention, there are provided compositions comprising a human Myt-1 kinase polynucleotide or an antisense sequence to this polynucleotide or a human Myt-1 kinase polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a human Myt-1 kinase polynucleotide for expression of a human Myt-1 kinase polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of human Myt-1 kinase.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

Brief Description of the Drawings

The following drawings depict certain embodiments of the invention. They are illustrative only and do not limit the invention otherwise disclosed herein.

Figure 1 shows the partial nucleotide sequence of human Myt-1 kinase (SEQ ID NO 1).

Figure 2 shows the deduced amino acid sequence of human Myt-1 kinase (SEQ ID NO 2).

Figure 3 shows a comparison between the deduced amino acid sequence of human Myt-1 kinase (SEQ ID NO 2) and *Xenopus* Myt-1 kinase (SEQ ID NO 3).

Glossary

The following illustrative explanations are provided to facilitate understanding of certain terms used frequently herein, particularly in the examples. The explanations are provided as a convenience and are not meant to limit the invention.

"Digestion" of DNA refers to catalytic cleavage of a DNA with an enzyme that acts only at certain sequences in the DNA. The various restriction enzymes referred to herein are commercially available and their reaction conditions, cofactors, and other requirements for use are known and routine to the skilled artisan.

For analytical purposes, typically 1 microgram of plasmid or DNA fragment is digested with about 2 units of enzyme in about 20 microliters of reaction buffer. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 micrograms of DNA are digested with 20 to 250 units of enzyme in proportionately larger volumes.

Appropriate buffers and substrate amounts for particular restriction enzymes are described in standard laboratory manuals, such as those referenced below, and are specified by commercial suppliers.

Incubation times of about 1 hour at 37°C are ordinarily used, but conditions may vary in accordance with standard procedures, the supplier's instructions and the particulars of the reaction. After digestion, reactions may be analyzed, and fragments may be purified by electrophoresis through an agarose or polyacrylamide gel, using well known methods that are routine for those skilled in the art.

"Genetic element" generally means a polynucleotide comprising a region that encodes a polypeptide or a region that regulates replication, transcription, translation or other processes important to expression of the polypeptide in a host cell, or a polynucleotide comprising both a region that encodes a polypeptide and a region operably linked thereto that regulates expression.

Genetic elements may be comprised within a vector that replicates as an episomal element, that is, as a molecule

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physically independent of the host cell genome. They may be comprised within mini-chromosomes, such as those that arise during amplification of transfected DNA by methotrexate selection in eukaryotic cells. Genetic elements also may be comprised within a host cell genome, not in their natural state but, rather, following manipulation such as isolation, cloning and introduction into a host cell in the form of purified DNA or in a vector, among others.

"Isolated" means altered "by the hand of man" from its natural state, i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living animal in its natural state is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs.

As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the polynucleotides and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of polynucleotides or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein, remain isolated polynucleotides or polypeptides within the meaning of that term as it is employed herein.

"Ligation" refers to the process of forming phosphodiester bonds between two or more polynucleotides, which most often are double stranded DNAs. Techniques for ligation are well known to the art and protocols for ligation are described in standard laboratory manuals and references, such as, for instance, Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, hereinafter referred to as Sambrook *et al.*

"Oligonucleotide(s)" refers to relatively short polynucleotides. Often the term refers to single-stranded deoxyribonucleotides, but it can refer as well to single- or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs, among others.

Oligonucleotides, such as single-stranded DNA probe oligonucleotides, are often synthesized by chemical methods, such as those implemented on automated oligonucleotide synthesizers. However, oligonucleotides can be made by a variety of other methods, including *in vitro* recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

Initially, chemically synthesized DNAs typically are obtained without a 5' phosphate. The 5' ends of such oligonucleotides are not substrates for phosphodiester bond formation by ligation reactions that employ DNA ligases typically used to form recombinant DNA molecules. Where ligation of such oligonucleotides is desired, a phosphate can be added by standard techniques, such as those that employ a kinase and ATP.

The 3' end of a chemically synthesized oligonucleotide generally has a free hydroxyl group and, in the presence of a ligase, such as T4 DNA ligase, will readily form a phosphodiester bond with a 5' phosphate of another polynucleotide, such as another oligonucleotide. As is well known, this reaction can be prevented selectively, where desired, by removing the 5' phosphates of the other polynucleotide(s) prior to ligation.

"Plasmids" are genetic elements that are stably inherited without being a part of the chromosome of their host cell. They may be comprised of DNA or RNA and may be linear or circular. Plasmids code for molecules that ensure their replication and stable inheritance during cell replication and may encode products of considerable medical, agricultural and environmental importance. For example, they code for toxins that greatly increase the virulence of pathogenic bacteria. They can also encode genes that confer resistance to antibiotics. Plasmids are widely used in molecular biology as vectors used to clone and express recombinant genes. Plasmids generally are designated herein by a lower case "p" preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill may readily construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily apparent to those of skill from the present disclosure.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide, as used herein, refers to triple-stranded regions comprising RNA or DNA or both

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RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term polynucleotide also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are polynucleotides as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including *inter alia* simple and complex cells.

"Polypeptides" as used herein includes all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textbooks and other publications in the art. In this context, the term is used herein to refer to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types.

It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes, such as processing and other post-translational modifications, or by chemical modification techniques which are well known to the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and thus are well known to those of skill in the art. Known modifications which may be present in polypeptides of the present invention include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, including glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, are described in most basic texts, such as PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993. Detailed reviews are also available on this subject. See, e.g., Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pages 1-12 in POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth. Enzymol.*, 1990, 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann. N.Y. Acad. Sci.*, 1992, 663:48-62.

It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural processes and by entirely synthetic methods, as well.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli*, prior to processing, almost invariably will be N-formylmethionine.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell's posttranslational modification capability and the modification signals present in the polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial hosts such as *E. coli*. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to express efficiently mammalian proteins having the native patterns of glycosylation, *inter alia*. Similar considerations apply to other modifications.

It will be appreciated that the same type of modification may be present in the same or varying degrees at several

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sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

"Variant(s)" of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide, respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail.

Variants include polynucleotides that differ in nucleotide sequence from another, reference polynucleotide. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

As noted below, changes in the nucleotide sequence of the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type, a variant will encode a polypeptide with the same amino acid sequence as the reference. As also noted below, changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below.

Variants also include polypeptides that differ in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many regions, identical.

A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

"Fusion protein" as the term is used herein, is a protein encoded by two, often unrelated, fused genes or fragments thereof. EP-A-0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified. Accordingly, it may be desirable to link two components of the fusion protein with a chemically or enzymatically cleavable linking region. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for use in high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, 1995, 8:52-58, and K. Johanson *et al.*, *The Journal of Biological Chemistry*, 1995, 270(16):9459-9471.

Thus, this invention also relates to genetically engineered soluble fusion proteins comprised of human Myt-1 kinase or a portion thereof, and of various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In one embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. This invention further relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for diagnosis and therapy. A further aspect of the invention relates to polynucleotides encoding such fusion proteins.

Membrane bound proteins are particularly useful in the formation of fusion proteins. Such proteins are generally characterized as possessing three distinct structural regions, an extracellular domain, a transmembrane domain, and a cytoplasmic domain. This invention contemplates the use of one or more of these regions as components of a fusion protein. Examples of such fusion protein technology can be found in WO94/29458 and WO94/22914.

"Binding molecules" (or otherwise called "interaction molecules" or "polypeptide component factors") refer to molecules, including receptors, that specifically bind to or interact with polypeptides of the present invention. Such binding molecules are a part of the present invention if they [illegible] naturally occurring, such as antibodies and antibody-derived reagents that bind specifically to polypeptides of the invention.

As known in the art, "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Moreover, also known in the art is "identity", which means the degree of sequence relatedness between two polypeptides or two polynucleotide sequences, as determined by the identity of the match between two strings of such sequences. Both identity and similarity can be readily calculated (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). There exist a number of methods to measure identity between two polynucleotide or polypeptide

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sequences and the terms "identity" and "similarity" are well known to skilled artisans (Carillo H and Lipton D *SIAM J Applied Math* 1988 *48* 1073). Methods commonly employed to determine identity or similarity between two sequences include but are not limited to those disclosed in Guide to Huge Computers Martin J Bishop ed Academic Press San Diego 1994 and Carillo H and Lipton D *SIAM J Applied Math* 1988 *48* 1073 Preferred methods to determine identity are designed to give the largest match between the two sequences tested Methods to determine identity and similarity are also codified in computer programs Preferred computer program methods to determine identity and similarity between two sequences include but are not limited to GCG program package (Devereux J *et al* *Nucleic Acids Research* 1984 *12* 1) 387) BLASTP BLASTN FASTA (Atschul S F *et al* *J Molec Biol* 1990 *215* 403)

Detailed Description of the Invention

The present invention relates to novel human Myt-1 kinase polypeptides and polynucleotides among other things as described in greater detail below In particular the invention relates to polypeptides and polynucleotides of a novel human Myt-1 kinase which is related by amino acid sequence homology to *Xenopus* Myt-1 kinase The invention relates especially to human Myt-1 kinase having the nucleotide and amino acid sequences set out in Figures 1 and 2

Polynucleotides

In accordance with one aspect of the present invention there are provided isolated polynucleotides which encode the human Myt-1 kinase polypeptide having the deduced amino acid sequence of Figure 2

Using the information provided herein such as the polynucleotide sequence set out in Figure 1 a polynucleotide of the present invention encoding human Myt-1 kinase may be obtained using standard cloning and screening procedures Illustrative of the invention the polynucleotide set out in Figure 1 was discovered in a cDNA library derived from cells of a chronic lymphocytic leukemia cell line using the expressed sequence tag (EST) analysis (Adams M D *et al Science* (1991) *252* 1651-1656 Adams M D *et al* *Nature* (1992) *355* 632-634 Adams M D *et al Nature* (1995) 377 Supp 3-174) This partial clone represents approximately 87% of the putative full length clone based upon the assumption that human Myt-1 gene is the same size as *Xenopus* Myt-1 Other partial length clones have been identified from breast cancer bone marrow and testes libraries

Human Myt-1 kinase of the invention is structurally related to other proteins of the Wee-1 family of kinases as shown by the results of sequencing the cDNA sequence set out in Figure 1 and also SEQ ID NO 1 It contains an open reading frame encoding a protein of approximately 479 amino acids Human Myt-1 kinase has 69 5% amino acid similarity (50 5% identity) to the *Xenopus* Myt-1 kinase The clone encodes the 5 conserved amino acids representative of the distinct kinase domain of the Wee-1 kinase family It also contains a putative transmembrane domain consistent with the membrane localization of the *Xenopus* Myt-1 which is a type II transmembrane protein The c-terminal region of this human clone has several potential phosphorylation sites which are believed to be involved in regulation of Myt

Polynucleotides of the present invention may be in the form of RNA such as mRNA or in the form of DNA including for instance cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof The DNA may be double-stranded or single-stranded Single-stranded DNA may be the coding strand also known as the sense strand or it may be the non-coding strand also referred to as the anti-sense strand

The coding sequence which encodes the polypeptide may be identical to the coding sequence of the polynucleotide shown in Figure 1 SEQ ID NO 1 It also may be a polynucleotide with a different sequence which as a result of the redundancy (degeneracy) of the genetic code also encodes the polypeptide of Figure 2 SEQ ID NO 2

Polynucleotides of the present invention which encode the polypeptide of Figure 2 may include but are not limited to the coding sequence for the mature polypeptide by itself the coding sequence for the mature polypeptide and additional coding sequences and the coding sequence of the mature polypeptide with or without the aforementioned additional coding sequences together with additional non-coding sequences Examples of additional coding sequence include but are not limited to sequences encoding a leader or secretory sequence such as a pre- or pro- or prepro-protein sequence Examples of additional non-coding sequences include but are not limited to introns and non-coding 5' and 3' sequences such as the transcribed non-translated sequences that play a role in transcription and mRNA processing including splicing and polyadenylation signals for example for ribosome binding and stability of mRNA Coding sequences which provide additional functionalities may also be incorporated into the polypeptide Thus for instance the polypeptide may be fused to a marker sequence such as a peptide which facilitates purification of the fused polypeptide In certain preferred embodiments of this aspect of the invention the marker sequence is a hexa-histidine peptide such as that provided in the pQE vector [illegible] described in Gentz *et al* *Proc Natl Acad Sci USA* 1989 *86* 821-824 for instance hexa-histidine provides convenient purification of the fusion protein In other embodiments the marker sequence is a HA tag [illegible] corresponds to an epitope derived of influenza hemagglutinin protein which has been described by Wils[illegible] 1984 *37* 767 for instance Many other such

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tags are commercially available

In accordance with the foregoing, the term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include, by virtue of the redundancy of the genetic code, any sequence encoding a polypeptide of the present invention, particularly the human Myt-1 kinase having the amino acid sequence set out in Figure 2, SEQ ID NO 2. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by introns) together with additional regions, that also may contain coding and/or non-coding sequences.

The present invention further relates to variants of the herein above described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 2. A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Among the particularly preferred embodiments of the invention in this regard are polynucleotides encoding polypeptides having the amino acid sequence of human Myt-1 kinase set out in Figure 2, variants, analogs, derivatives and fragments thereof, and fragments of the variants, analogs and derivatives.

Further particularly preferred in this regard are polynucleotides encoding human Myt-1 kinase variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence of the human Myt-1 kinase polypeptide of Figure 2 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the human Myt-1 kinase. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polynucleotides encoding polypeptides having the amino acid sequence of Figure 2, without substitutions.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical to a polynucleotide encoding the human Myt-1 kinase polypeptide having the amino acid sequence set out in Figure 2, and polynucleotides which are complementary to such polynucleotides. More preferred are polynucleotides that comprise a region that is at least 80% identical to a polynucleotide encoding the human Myt-1 kinase polypeptide and polynucleotides complementary thereto. Polynucleotides at least 90% identical to the same are particularly preferred, and those with at least 95% are more particularly preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are more highly preferred, with at least 99% being the most preferred.

Particularly preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding human Myt-1 kinase and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the human Myt-1 kinase gene. Such probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

For example, the coding region of the human Myt-1 kinase gene may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of human cDNA, genomic DNA or mRNA to determine the members of the library to which the probe hybridizes to.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to human disease, as further discussed herein relating to polynucleotide assays.

The polynucleotides may encode a polypeptide which is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may facilitate protein trafficking, may prolong or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in situ*, the additional amino acids may be proc

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essed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In summary, a polynucleotide of the present invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Polypeptide

The present invention further relates to a human Myt-1 kinase polypeptide which has the deduced amino acid sequence of Figure 2 (SEQ ID NO:2). The invention also relates to fragments, analogs and derivatives of thereof. The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 2, mean a polypeptide which retains essentially the same biological function or activity as such polypeptide. (i.e. functions as a Myt-1 kinase or retains the ability to bind any receptors or binding molecules even though the polypeptide does not function as the enzyme). Thus, an analog includes, for example, a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. In certain preferred embodiments, it is a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 2 may be: (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; (ii) one in which one or more of the amino acid residues includes a substituent group; (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of human Myt-1 kinase set out in Figure 2 as SEQ ID NO:2, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Further particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of human Myt-1 kinase, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments which retain the activity/function of this enzyme.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between the amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of the human Myt-1 kinase polypeptide of Figure 2 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the enzyme. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having the amino acid sequence of Figure 2, SEQ ID NO:2, without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 70% identity to the polypeptide of SEQ ID NO:2 and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of SEQ ID NO:2 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for pro-

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ducing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention. Fragments may be "freestanding," i.e., not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a human Myt-1 kinase polypeptide of the present comprised within a precursor polypeptide designed for expression in a host and having heterologous pre- and pro-polypeptide regions fused to the amino terminus of the human Myt-1 kinase fragment and an additional region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from human Myt-1 kinase.

As representative examples of polypeptide fragments of the invention, there may be mentioned those which have from about 5-15, 10-20, 15-40, 30-55, 41-75, 41-80, 41-90, 50-100, 75-100, 90-115, 100-125, and 110-113 amino acids in length.

In this context "about" includes the particularly recited range and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either extreme or at both extremes. For instance, about 40-90 amino acids in this context means a polypeptide fragment of 40 plus or minus several, a few, 5, 4, 3, 2 or 1 amino acid residues to 90 plus or minus several a few, 5, 4, 3, 2 or 1 amino acid residues, i.e., ranges as broad as 40 minus several amino acids to 90 plus several amino acids to as narrow as 40 plus several amino acids to 90 minus several amino acids. Highly preferred in this regard are the recited ranges plus or minus as many as 5 amino acids at either or at both extremes. Particularly highly preferred are the recited ranges plus or minus as many as 3 amino acids at either or at both the recited extremes. Especially particularly highly preferred are ranges plus or minus 1 amino acid at either or at both extremes or the recited ranges with no additions or deletions. Most highly preferred of all in this regard are fragments from about 5-15, 10-20, 15-40, 30-55, 41-75, 41-80, 41-90, 50-100, 75-100, 90-115, 100-125, and 110-113 amino acids long.

Among especially preferred fragments of the invention are truncation mutants of human Myt-1 kinase. Truncation mutants include human Myt-1 kinase polypeptides having the amino acid sequence of Figure 2, or of variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Particularly preferred fragments of the membrane bound enzymes of this invention include soluble forms of the enzyme comprising the extracellular domain without its attendant transmembrane and cytoplasmic domains or transmembrane region deletions resulting in an enzyme in which the extracellular domain is fused directly to the cytoplasmic domain. See for example, published PCT application number WO94/03620. Alternatively, fragments of the present invention include deletion of the transmembrane region only and retention of at least part of the cytoplasmic domain itself or fusion with at least part of an alternate cytoplasmic domain as described in WO96 04382. Fragments having the size ranges set out above are also preferred embodiments of truncation fragments, which are especially preferred among fragments generally.

Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of human Myt-1 kinase. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of human Myt-1 kinase.

Among highly preferred fragments in this regard are those that comprise regions of human Myt-1 kinase that combine several structural features, such as several of the features set out above. Such regions may be comprised within a larger polypeptide or may be by themselves a preferred fragment of the present invention, as discussed above. It will be appreciated that [illegible] the ranges set out above regarding fragments in general.

Further preferred regions are those that mediate activities of the Myt-1 enzyme. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of human Myt-1 kinase, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Highly preferred in this regard are fragments that contain regions that are homologs in sequence, or in position, or in both sequence and position to active regions of related polypeptides, such as human Myt-1 kinase. Among particularly preferred fragments in these regards are truncation mutants, as discussed above, or fragments comprising cytoplasmic, transmembrane or extracellular domains.

It will be appreciated that the invention also relates to, among others, polynucleotides encoding the aforementioned fragments, polynucleotides that hybridize to polynucleotides encoding the fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode the

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fragments. In these regards, preferred polynucleotides are those that correspond to the preferred fragments, as discussed above.

Vectors, host cells, expression

The present invention also relates to vectors which contain polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. For instance, polynucleotides may be introduced into host cells using well known techniques of infection, transduction, transfection, transvection and transformation. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

Thus, for instance, polynucleotides of the invention may be transfected into host cells with another, separate, polynucleotide encoding a selectable marker, using standard techniques for co-transfection and selection in, for instance, mammalian cells. In this case the polynucleotides generally will be stably incorporated into the host cell genome.

Alternatively, the polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. The vector construct may be introduced into host cells by the aforementioned techniques. Generally, a plasmid vector is introduced as DNA in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. Electroporation may also be used to introduce polynucleotides into a host. If the vector is a virus, it may be packaged *in vitro* or introduced into a packaging cell and the packaged virus may be transduced into cells. A wide variety of techniques suitable for making polynucleotides and for introducing polynucleotides into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length in Sambrook *et al.* which is merely illustrative of the many laboratory manuals that detail these techniques.

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single- or double-stranded phage vector, or a single- or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. The vectors, in the case of phage and viral vectors may also be and preferably are introduced into cells as packaged or encapsidated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors are either supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific expression. Particularly preferred among inducible vectors are vectors that can be induced to express a protein by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

The engineered host cells can be cultured in conventional nutrient media, which may be modified as appropriate for, *inter alia*, activating promoters, selecting transformants or amplifying genes. Culture conditions, such as temperature, pH and the like, previously used with the host cell selected for expression, generally will be suitable for expression of polypeptides of the present invention as will be apparent to those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, bacteriophages, yeast episomes, yeast chromosomal elements, and viruses such as baculoviruses, papova viruses, SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, cosmids and phagemids. Generally, any vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques. In general, a DNA sequence for expression is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction endonucleases and then joining the restriction fragments together using T4 DNA ligase. Procedures for restriction and ligation that can be used to this end are well known and routine to those of skill. Suitable procedures in this regard, and for constructing expression vectors using alternative techniques which also are well known and routine to those skilled in the art, are set forth in great detail in Sambrook *et al.*

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s)

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including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known promoters. It will be understood that numerous other promoters useful in this aspect of the invention are well known and may be routinely employed by those of skill in the manner illustrated by the discussion and the examples herein.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate, as well as engender, expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription. Examples include repressor binding sites and enhancers, among others.

Vectors for propagation and expression generally will include selectable markers. Selectable marker genes provide a phenotypic trait for selection of transformed host cells. Preferred markers include, but are not limited to, dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing *E. coli* and other bacteria. Such markers may also be suitable for amplification. Alternatively, the vectors may contain additional markers for this purpose.

The vector containing a selected polynucleotide sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable for expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure to routinely select a host for expressing a polypeptide in accordance with this aspect of the present invention.

More particularly, the present invention also includes recombinant constructs, such as expression constructs, comprising one or more of the sequences described above. The constructs comprise a vector, such as a plasmid or viral vector, into which such a sequence of the invention has been inserted, in a forward or reverse orientation. In certain preferred embodiments in this regard, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and there are many commercially available vectors suitable for use in the present invention.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5, available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG, available from Stratagene; and pSVK3, pBPV, pMSG and pSVL, available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("CAT") transcription unit, downstream of a restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the CAT gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available. Two examples of such vectors are pKK232-8 and pCM7. Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that may be readily obtained by the foregoing method using a reporter gene.

Among known bacterial promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E. coli* lac and lacZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR, PL promoters and the trp promoter.

Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous Sarcoma Virus("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for construction of expression vectors, introduction of the vector into the host and expression in the host are routine skills in the art.

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The present invention also relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, a lower eukaryotic cell, such as a yeast cell, or a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals.

Constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook *et al*.

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells following exposure to the vector. Among suitable promoters are those derived from the genes that encode glycolytic enzymes such as 3-phosphoglycerate kinase ("PGK"), α-factor, acid phosphatase, and heat shock proteins, among others. Selectable markers include the ampicillin resistance gene of *E. coli* and the trp1 gene of *S. cerevisiae*.

Transcription of DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually from about 10 to 300 bp, that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

A polynucleotide of the invention encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 5' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiation codon. Also, generally, there will be a translation stop codon at the end of the polypeptide and a poly-adenylation signal and transcription termination signal appropriately disposed at the 3' end of the transcribed region.

Appropriate secretion signals may be incorporated into the expressed polypeptide for secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. The signals may be endogenous to the polypeptide or heterologous.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for example, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell during purification or subsequent handling and storage. A region may also be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

Suitable prokaryotic hosts for propagation, maintenance or expression of polynucleotides and polypeptides in accordance with the invention include *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium*. Various species of *Pseudomonas*, *Streptomyces*, and *Staphylococcus* are also suitable hosts in this regard. Moreover, many other hosts also known to those of skill may be employed in this regard.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). In these vectors, the pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain, the host strain is grown to an appropriate cell density. Where the selected promoter is inducible, it is induced by appropriate means (e.g., temperature shift or exposure to chemical inducer) and cells are cultured for an additional period. Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-

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thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art.

Various mammalian cell culture systems can be employed for expression as well. Examples of mammalian expression systems include the C127, 3T3, CHO, HeLa, human kidney 293 and BHK cell lines, and the COS-7 line of monkey kidney fibroblasts, described by Gluzman *et al.*, *Cell*, 1981, *23*:175.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are necessary for expression. In certain preferred embodiments, DNA sequences derived from the SV40 splice sites and the SV40 polyadenylation sites are used for required non-transcribed genetic elements.

The human Myt-1 kinase polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Polypeptides of the present invention include naturally purified polypeptides, polypeptides produced by chemical synthetic procedures, and polypeptides produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or non-glycosylated. In addition, polypeptides of the invention may include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Human Myt-1 kinase polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of the enzyme. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are illustrated further by the following discussion.

Polynucleotide assays

This invention is also related to the use of human Myt-1 kinase polynucleotides to detect complementary polynucleotides for use, for example, as a diagnostic reagent. Detection of a mutated form of human Myt-1 kinase associated with a dysfunction will provide a diagnostic tool that can add to or define diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of human Myt-1 kinase. Individuals carrying mutations in the human Myt-1 kinase gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. PCR (Saiki *et al.*, *Nature*, 1986, 324:163-166) RNA or cDNA may also be used in similar fashion. As an example, PCR primers complementary to the nucleic acid encoding human Myt-1 kinase can be used to identify and analyze human Myt-1 kinase expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled Myt-1 kinase RNA or radiolabeled Myt-1 kinase antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations may also be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or other amplification methods. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, *Science*, 1985, *230*:1242).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, *Proc. Natl. Acad. Sci. USA*, 1985, *85*:4397-4401).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase pro-

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tection chemical cleavage direct DNA sequencing or the use of restriction enzymes (e.g. restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In accordance with a further aspect of the invention, there is provided a process for diagnosing or determining a susceptibility to hyperproliferative diseases including cancers such as leukemia, solid tumors and metastases, chronic inflammatory proliferative disease such as psoriasis and rheumatoid arthritis, proliferative cardiovascular diseases such as restenosis, proliferative ocular disorders such as diabetic retinopathy and macular degeneration, and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas, among others. A mutation in the human Myt-1 kinase gene may be indicative of a susceptibility to hyperproliferative diseases including cancers such as leukemia, solid tumors and metastases, chronic inflammatory proliferative disease such as psoriasis and rheumatoid arthritis, proliferative cardiovascular diseases such as restenosis, proliferative ocular disorders such as diabetic retinopathy and macular degeneration, and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas, among others, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Thus, for example, the assay may be employed to determine a mutation in a human Myt-1 kinase gene, as herein described, such as a substitution, deletion, truncation, insertion, frame shift, etc., with such mutation being indicative of a susceptibility to a hyperproliferative disease, among others.

The invention provides a process for diagnosing diseases, particularly hyperproliferative diseases including cancers such as leukemia, solid tumors and metastases, chronic inflammatory proliferative disease, such as psoriasis and rheumatoid arthritis, proliferative cardiovascular diseases such as restenosis, proliferative ocular disorders such as diabetic retinopathy and macular degeneration, and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas, among others, comprising determining from a sample derived from a patient an abnormally decreased or increased level of expression of polynucleotide having the sequence of Figure 1, SEQ ID NO: 1. Decreased or increased expression of polynucleotide can be measured using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Chromosome assays

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, because primers that span more than one exon could complicate the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, subcalibration can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can be used similarly to map to the chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNAs as short as 50 to 60 bases. For a review of this technique, see Verma *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES, PERGAMON PRESS, NEW YORK, 1988.

As an example of how this technique is performed, human Myt-1 kinase DNA is digested and purified with a QIAEX II DNA purification kit (Qiagen, Inc., Chatsworth, CA) and ligated to Super Cos I cosmid vector (Stratagene, La Jolla, CA). DNA is purified using a Qiagen Plasmid Purification kit (Qiagen, Inc., Chatsworth, CA) and 1 mg is labeled by nick translation in the presence of Biotin-dATP using a BioNick Labeling kit (GibcoBRL, Life Technologies Inc., Gaithersburg, MD). Biotinylation is detected with GENE-TECT Detection System (Clontech Laboratories, Inc., Palo Alto, CA). *In situ* hybridization is performed on slides using ONCOR Light Hybridization Kit (Oncor, Gaithersburg, MD) to detect single copy sequences on metaphase chromosomes. Peripheral blood of normal donors is cultured for three days in RPMI 1640 supplemented with 20% FCS, 3% PHA and penicillin-streptomycin, synchronized with 10^{-7} M methotrexate for 17 hours, and washed twice with unsupplemented RPMI. Cells are then incubated with 10^{-3} M thymidine for 7 hours.

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The cells are arrested in metaphase after a 20 minute incubation with colcemid (0.5 µg/ml) followed by hypotonic lysis in 75 mM KCl for 15 minutes at 37°C. Cell pellets are then spun out and fixed in Carnoy's fixative (3:1 methanol/acetic acid).

Metaphase spreads are prepared by adding a drop of the suspension onto slides and air drying the suspension. Hybridization is performed by adding 100 ng of probe suspended in 10 ml of hybridization mix (50% formamide, 2xSSC, 10% dextran sulfate) with blocking human placental DNA (1 µg/ml). Probe mixture is denatured for 10 minutes in a 70°C water bath and incubated for 1 hour at 37°C before placement on a prewarmed (37°C) slide, previously denatured in 70% formamide/2xSSC at 70°C, dehydrated in ethanol series, and chilled to 4°C.

Slides are incubated for 16 hours at 37°C in a humidified chamber. Slides are washed in 50% formamide/2xSSC for 10 minutes at 41°C and 2xSSC for 7 minutes at 37°C. Hybridization probe is detected by incubation of the slides with FITC-Avidin (ONCOR, Gaithersburg, MD) according to the manufacturer's protocol. Chromosomes are counterstained with propidium iodine suspended in mounting medium. Slides are visualized using a Leitz ORTHOPLAN 2-epi-fluorescence microscope and five computer images are taken using a Imagenetics Computer and MacIntosh printer.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

It is then necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes, assuming 1 megabase mapping resolution and one gene per 20 kb.

Polypeptide assays

The present invention also relates to diagnostic assays for detecting levels of human Myt-1 kinase protein in cells and tissues. Such assays may be quantitative or qualitative. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of human Myt-1 kinase protein compared to normal control tissue samples may be used to detect the presence of hyperproliferative diseases, including cancers, such as leukemia, solid tumors and metastases, chronic inflammatory proliferative disease, such as psoriasis and rheumatoid arthritis, proliferative cardiovascular diseases, such as restenosis, proliferative ocular disorders, such as diabetic retinopathy and macular degeneration, and benign hyperproliferative diseases, such as benign prostatic hypertrophy and hemangiomas, among others. Assay techniques that can be used to determine levels of a protein, such as a human Myt-1 kinase protein of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and enzyme linked immunosorbent assays (ELISAs). Among these, ELISAs are frequently preferred. An ELISA assay initially comprises preparing an antibody specific to human Myt-1 kinase, preferably a monoclonal antibody. In addition, a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached to a detectable reagent such as a radioactive, fluorescent or enzymatic reagent, in this example, horseradish peroxidase enzyme.

To carry out an ELISA, a sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. The monoclonal antibody is then incubated in the dish during which time the monoclonal antibodies attach to any human Myt-1 kinase proteins attached to the polystyrene dish. Unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to Myt-1 kinase protein. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate, are then added to the dish. Immobilized peroxidase linked antibody, specific for human Myt-1 kinase (primary and secondary antibodies), produces a colored reaction product. The amount of color developed in a given time period indicates the amount of human Myt-1 kinase protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may also be employed wherein antibodies specific to human Myt-1 kinase attached to a solid support and labeled human Myt-1 kinase and a sample derived from the host are passed over the solid support. The amount of detected label attached to the solid support can then be correlated to a quantity of human Myt-1 kinase in the sample.

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Antibodies

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of a Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against polypeptides corresponding to a sequence of the present invention can be obtained by various means well known to those of skill in the art. For example, in one embodiment, the polypeptide is directly injected into an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this embodiment, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies binding the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from tissues expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature*, 1975, *256*, 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today*, 1983, *4*, 72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pages 77-96, Alan R. Liss, Inc., 1985).

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Antibodies against human Myt-1 kinase may also be employed to inhibit hyperproliferative diseases including cancers, such as leukemia, solid tumors and metastases; chronic inflammatory proliferative disease, such as psoriasis and rheumatoid arthritis; proliferative cardiovascular diseases, such as restenosis; proliferative ocular disorders, such as diabetic retinopathy and macular degeneration; and benign hyperproliferative diseases, such as benign prostatic hypertrophy and hemangiomas, among others.

Myt-1 kinase binding molecules and assays

Human Myt-1 kinase can also be used to isolate proteins which interact with it; this interaction can be a target for interference. Inhibitors of protein-protein interactions between human Myt-1 kinase and other factors could lead to the development of pharmaceutical agents for the modulation of human Myt-1 kinase activity.

Thus, this invention also provides a method for identification of binding molecules to human Myt-1 kinase. Genes encoding proteins for binding molecules to human Myt-1 kinase can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Such methods are described in many laboratory manuals such as, for instance, Coligan *et al.*, CURRENT PROTOCOLS IN IMMUNOLOGY 1, Chapter 5, 1991.

For example, the yeast two-hybrid system provides methods for detecting the interaction between a first test protein and a second test protein *in vivo*, using reconstitution of the activity of a transcriptional activator. The method is disclosed in U.S. Patent No. 5,283,173. Reagents are available from Clontech and Stratagene. Briefly, human Myt-1 kinase cDNA is fused to a Gal4 transcription factor DNA binding domain and expressed in yeast cells. cDNA library members obtained from cells of interest are fused to a transactivation domain of Gal4. cDNA clones which express proteins which can interact with human Myt-1 kinase will lead to reconstitution of Gal4 activity and transactivation of expression of a reporter gene such as Gal4-lacZ.

An alternative method involves screening of λgt11, λZAP (Stratagene) or equivalent cDNA expression libraries with recombinant human Myt-1 kinase. Recombinant human Myt-1 kinase protein or fragments thereof are fused to small peptide tags such as FLAG, HSV or GST. The peptide tags can possess convenient phosphorylation sites for a kinase such as heart muscle creatine kinase or they can be biotinylated. Recombinant human Myt-1 kinase can be phosphorylated with ^{32}[P] or used unlabeled and detected with streptavidin or antibodies against the tags. λgt11cDNA expression libraries are made from cells of interest and [illegible] incubated with the recombinant human Myt-1 kinase, washed and cDNA clones which interact with human Myt-1 [illegible] detected. Such methods are routinely used by skilled artisans. See, e.g., Sambrook *et al.*

Another method is the screening of a mammalian expression [illegible] this method, cDNAs are cloned into a vector between a mammalian promoter and polyadenylation site [illegible], transfected in COS or 293 cells. Forty-eight hours later, the binding protein is detected by incubation [illegible] cells with labeled human Myt-1 kinase.

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In a preferred embodiment the human Myt-1 kinase is iodinated and any bound human Myt-1 kinase is detected by autoradiography See Sims *et al*. *Science*. 1988. *241* 585-589 and McMahan *et al*. *EMBO J*. 1991 *10* 2821-2832 In this manner pools of cDNAs containing the cDNA encoding the binding protein of interest can be selected and the cDNA of interest can be isolated by further subdivision of each pool followed by cycles of transient transfection binding and autoradiography Alternatively the cDNA of interest can be isolated by transfecting the entire cDNA library into mammalian cells and panning the cells on a dish containing human Myt-1 kinase bound to the plate Cells which attach after washing are lysed and the plasmid DNA isolated amplified in bacteria and the cycle of transfection and panning repeated until a single cDNA clone is obtained See Seed *et al*. *Proc Natl Acad Sci USA*. 1987 *84* 3365 and Aruffo *et al*. *EMBO J*. 1987. *6* 3313 If the binding protein is secreted its cDNA can be obtained by a similar pooling strategy once a binding or neutralizing assay has been established for assaying supernatants from transiently transfected cells General methods for screening supernatants are disclosed in Wong *et al*. *Science*. 1985. *228* 810-815

Another method involves isolation of proteins interacting with human Myt-1 kinase directly from cells Fusion proteins of human Myt-1 kinase with GST or small peptide tags are made and immobilized on beads Biosynthetically labeled or unlabeled protein extracts from the cells of interest are prepared incubated with the beads and washed with buffer Proteins interacting with human Myt-1 kinase are eluted specifically from the beads and analyzed by SDS-PAGE Binding partner primary amino acid sequence data are obtained by microsequencing Optionally the cells can be treated with agents that induce a functional response such as tyrosine phosphorylation of cellular proteins An example of such an agent would be a growth factor or cytokine such as interleukin-2.

Another method is immunoaffinity purification Recombinant human Myt-1 kinase is incubated with labeled or unlabeled cell extracts and immunoprecipitated with anti-Myt-1 kinase antibodies The immunoprecipitate is recovered with protein A-Sepharose and analyzed by SDS-PAGE Unlabelled proteins are labeled by biotinylation and detected on SDS gels with streptavidin Binding partner proteins are analyzed by microsequencing Further standard biochemical purification steps known to those skilled in the art may be used prior to microsequencing

Yet another alternative method involves screening of peptide libraries for binding partners Recombinant tagged or labeled human Myt-1 kinase is used to select peptides from a peptide or phosphopeptide library which interact with human Myt-1 kinase Sequencing of the peptides leads to identification of consensus peptide sequences which might be found in interacting proteins

Agonists and antagonists - assays and molecules

The human Myt-1 kinase of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation (antagonists) of this enzyme

Examples of potential kinase antagonists include antibodies or in some cases oligonucleotides which bind to the enzyme but do not elicit a second messenger response such that the activity of the enzyme is prevented

Potential antagonists also include proteins which are closely related to human Myt-1 kinase i.e. a fragment of the enzyme which have lost enzymatic activity

A potential antagonist also includes an antisense construct prepared through the use of antisense technology Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA both methods of which are based on binding of a polynucleotide to DNA or RNA For example the 5' coding portion of the polynucleotide sequence which encodes for the mature polypeptides of the present invention is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription triple helix see Lee *et al*. *Nucl Acids Res*. 1979 *6* 3073 Cooney *et al*. *Science* 1988 *241* 456 and Dervan *et al*. *Science* 1991 *251* 1360 thereby preventing transcription and production of the human Myt-1 kinase The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the enzyme antisense see Okano *J Neurochem* (1991. *56* 560 Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression CRC Press Boca Raton FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA is expressed *in vivo* to inhibit production of human Myt-1 kinase

Another potential antagonist is a small molecule which binds to the enzyme for example at the active site thereby blocking substrate from binding to the enzyme so that normal biological activity is prevented Examples of small molecules include but are not limited to small peptides or peptide-like molecules

Potential antagonists also include soluble forms of human Myt-1 kinase e.g. fragments of the enzyme which bind to ligands thus preventing the ligand from interacting with membrane bound human Myt-1 kinase

The Myt-1 kinases are ubiquitous in the mammalian host and are responsible for many biological functions including many pathologies Accordingly it is desirous to find compounds and drugs which stimulate enzyme activity on the one hand and which can inhibit the function of Myt-1 kinase on the other hand

Antagonists for human Myt-1 kinase may be employed for a variety of therapeutic and prophylactic purposes for such hyperproliferative diseases or disorders as cancers such as leukemia solid tumors and metastases chronic

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inflammatory proliferative disease such as psoriasis and rheumatoid arthritis proliferative cardiovascular diseases such as restenosis proliferative ocular disorders such as diabetic retinopathy and macular degeneration and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas among others

This invention additionally provides a method of treating an abnormal condition where Myt-1 activity is involved in the abnormal conditions This method comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation of the enzyme or by inhibiting a second signal and thereby alleviating the abnormal condition For example blocking activity of Myt-1 in hyperproliferative cells with an antagonist would disrupt the timing of the cell cycle thus causing cells to divide before they are ready and resulting in cell death

The invention also provides a method of treating abnormal conditions related to an under-expression of human Myt-1 kinase and its activity which comprises administering to a subject a therapeutically effective amount of a compound which activates (agonist) the enzyme to thereby alleviate the abnormal condition

Compositions and Kits

The soluble form of human Myt-1 kinase and compounds which activate or inhibit such enzyme may be employed in combination with a suitable pharmaceutical carrier Such compositions comprise a therapeutically effective amount of the polypeptide or compound and a pharmaceutically acceptable carrier or excipient Such carriers include but are not limited to saline buffered saline dextrose water glycerol ethanol and combinations thereof The formulation should suit the mode of administration Selection of an appropriate carrier in accordance with the mode of administration is routinely performed by those skilled in the art

The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention

Administration

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds such as therapeutic compounds

The pharmaceutical compositions may be administered in any effective convenient manner including for instance administration by topical oral anal vaginal intravenous intraperitoneal intramuscular subcutaneous intranasal or intradermal routes among others

The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications In general the compositions are administered in an amount of at least about 10 µg/kg body weight In most cases they will be administered in an amount not in excess of about 8 mg/kg body weight per day Preferably in most cases the administered dose is from about 10 µg/kg to about 1 mg/kg body weight daily It will be appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication taking into account the indication its severity route of administration complicating conditions and the like

Gene therapy

Human Myt-1 kinase polynucleotides polypeptides agonists and antagonists that are polypeptides may be employed in accordance with the present invention by expression of such polypeptides in treatment modalities often referred to as "gene therapy"

Thus for example cells from a patient may be engineered with a polynucleotide such as a DNA or RNA to encode a polypeptide *ex vivo* The engineered cells can then be provided to a patient to be treated with the polypeptide In this embodiment cells may be engineered *ex vivo* for example by the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention Such methods are well-known in the art and their use in the present invention will be apparent from the teachings herein

Similarly cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by procedures known in the art For example a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector as discussed above The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest These producer cells may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo* These and other methods for administering a polypeptide of the present invention should be apparent to those skilled in the art from the teachings of the present invention

Retroviruses from which the retroviral plasmid vectors herein above mentioned may be derived include but are not limited to Moloney Murine Leukemia Virus Spleen Necrosis Virus Rous Sarcoma Virus Harvey Sarcoma Virus

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Avian Leukosis Virus, Gibbon Ape Leukemia Virus, Human Immunodeficiency Virus, Adenovirus, Myeloproliferative Sarcoma Virus, and Mammary Tumor Virus. In a preferred embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

Such vectors will include one or more promoters for expressing the polypeptide. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR, the SV40 promoter, and the human cytomegalovirus (CMV) promoter described in Miller *et al.*, *Biotechniques*, 1989, 7:980-990. Cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, RNA polymerase III, and β-actin promoters can also be used. Additional viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention will be placed under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs herein above described); the β-actin promoter; and human growth hormone promoters. The promoter may also be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, Y-2, Y-AM, PA12, T19-14X, VT-19-17-H2, YCRE, YCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, A., *Human Gene Therapy*, 1990, 1:5-14. The vector may be transduced into the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line will generate infectious retroviral vector particles, which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles may then be employed to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

EXAMPLES

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Certain terms used herein are explained in the foregoing glossary.

All examples are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook *et al.*

EXAMPLE 1: Protein Analysis

Samples are resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels. To analyze cdc2, the substrate of Myt-1 kinase, anti-cdc2 immunoblotting is performed with an affinity-purified rabbit polyclonal serum prepared against a synthetic peptide corresponding to residues 4-18 of cdc2 protein, as described by Mitarski *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 1991, 56:377-384. Following immunoblotting, the nitrocellulose filters are treated with ^{125}I-protein A. Autoradiography is performed with an intensifying screen at -70°C.

For peptide mapping, ^{32}P-labeled samples are resolved by SDS-PAGE, transferred to IMMOBILON-P (Millipore, Bedford, MA) and analyzed by autoradiography. Peptide mapping is performed in accordance with procedures described by Boyle et al., *Meth. Enzymol.*, 1991, 201:110-149. The ^{32}P-labeled tryptic digests are spotted onto 100 μM thin-layer cellulose plates and electrophoresed at pH 1.9 for 25 minutes at 1 kV. Chromatography in the second dimension is performed in phosphochrome buffer. Phosphoamino acid analysis is performed in accordance with procedures described by Boyle et al., *Meth. Enzymol.*, 1991, *201*:110-149.

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EXAMPLE 2: Shift Assays. Cyclin. and p13 Binding

To assay the activity of Myt-1 kinase a mobility shift of the substrate cdc2 is measured To assay the mobility of the cdc2 protein 30 µl aliquots of extracts (with or without added membranes) are incubated at room temperature for 30 minutes Phosphatase activity is then inhibited by addition of 0 5 mM sodium orthovanadate Glutathione-S-transferase sea urchin cyclin B (GST fusion protein) is then added and the incubation is continued for an additional 15 minutes Following the incubation the samples are rapidly frozen in liquid nitrogen for storage at -70°C

For processing samples are thawed by a 1 1 dilution in buffer containing 80 mM B-glycerophosphate 5 mM EDTA 2 mM sodium orthovanadate 0 1% Nonidet P-40 and 0 5 M NaCl Samples are either bound to glutathione agarose beads or p13-Sepharose and processed in accordance with procedures described by Smythe C and Newport J W *Cell* 1992 3 1 -27

EXAMPLE 3: H1 Assays

To assay the activity of cdc2 phosphorylation of histone H1 is followed in this assay recombinant GST-cyclin is added to interphase extracts in the presence or absence of added membranes and 2 µl of EB buffer containing 20 mM B-glycerophosphate pH 7 3 20 mM EGTA and 15 mM $MgCl_2$ Samples are frozen in liquid nitrogen and stored at -70°C The histone kinase activity is assayed in accordance with procedures described by Kornbluth *et al* *Mol Cell Biol* 1992 12 3216-3223

EXAMPLE 4: Salt and Detergent Extraction of Cell Membranes

Cell membranes are incubated on ice for 30 minutes with lysing buffer in various concentrations of KCl Cell membranes are pelleted by ultracentrifugation and then diluted 5-fold in lysis buffer and repelleted in 0 5 M sucrose The membranes are then added at 1 10 volume to buffer and vanadate and GST cyclin cdc2 kinase For detergent treatment membranes are incubated on ice for 15 minutes with detergent and lysing buffer Membranes are then pelleted by microcentrifugation for 30 minutes The pellets are resuspended in 5 volumes of lysis buffer containing 2 mM ATP 20 mM phosphocreatinine and 50 µg ml creatine kinase The pellet and supernatant fractions are incubated separately with GST cyclin/cdc2 complexes which are prepared in the absence of vanadate to allow phosphorylation of cdc protein kinase and Tyr^{15} and Thr^{14}

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF INVENTION: Human Myt-1 Kinase Clone

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property

(B) STREET: Two New Horizons Court

(C) CITY: Brentford

(D) COUNTY: Middlesex

(E) COUNTRY: GB

(F) POST CODE: TW8 9EP

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb STORAGE

(B) COMPUTER: IBM 486

(C) OPERATING SYSTEM: WINDOWS FOR WORKGROUPS

(D) SOFTWARE: MICROSOFT WORD

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: not yet assigned

(B) FILING DATE: Herewith

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

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(viii) ATTORNEY/AGENT INFORMATION:

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(B) GENERAL AUTHORISATION NUMBER: 5630

(C) REFERENCE/DOCKET NUMBER: ATG 50027

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(2) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1448

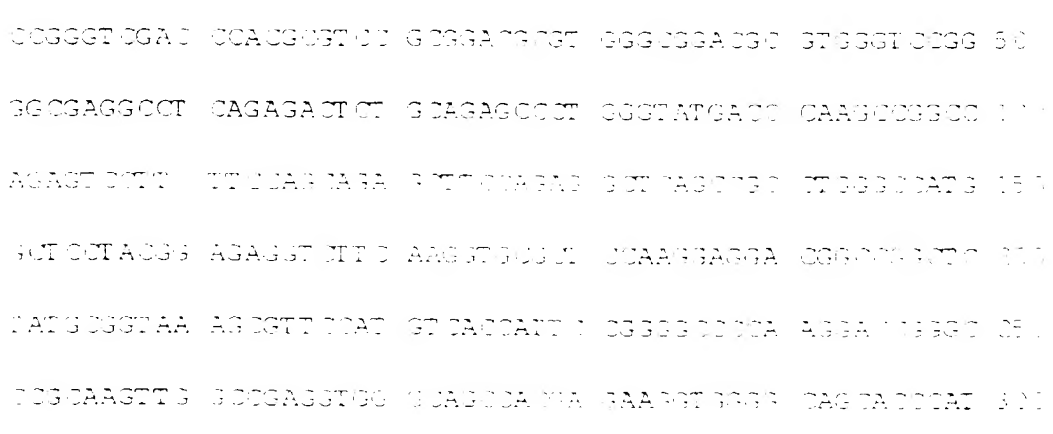
(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1



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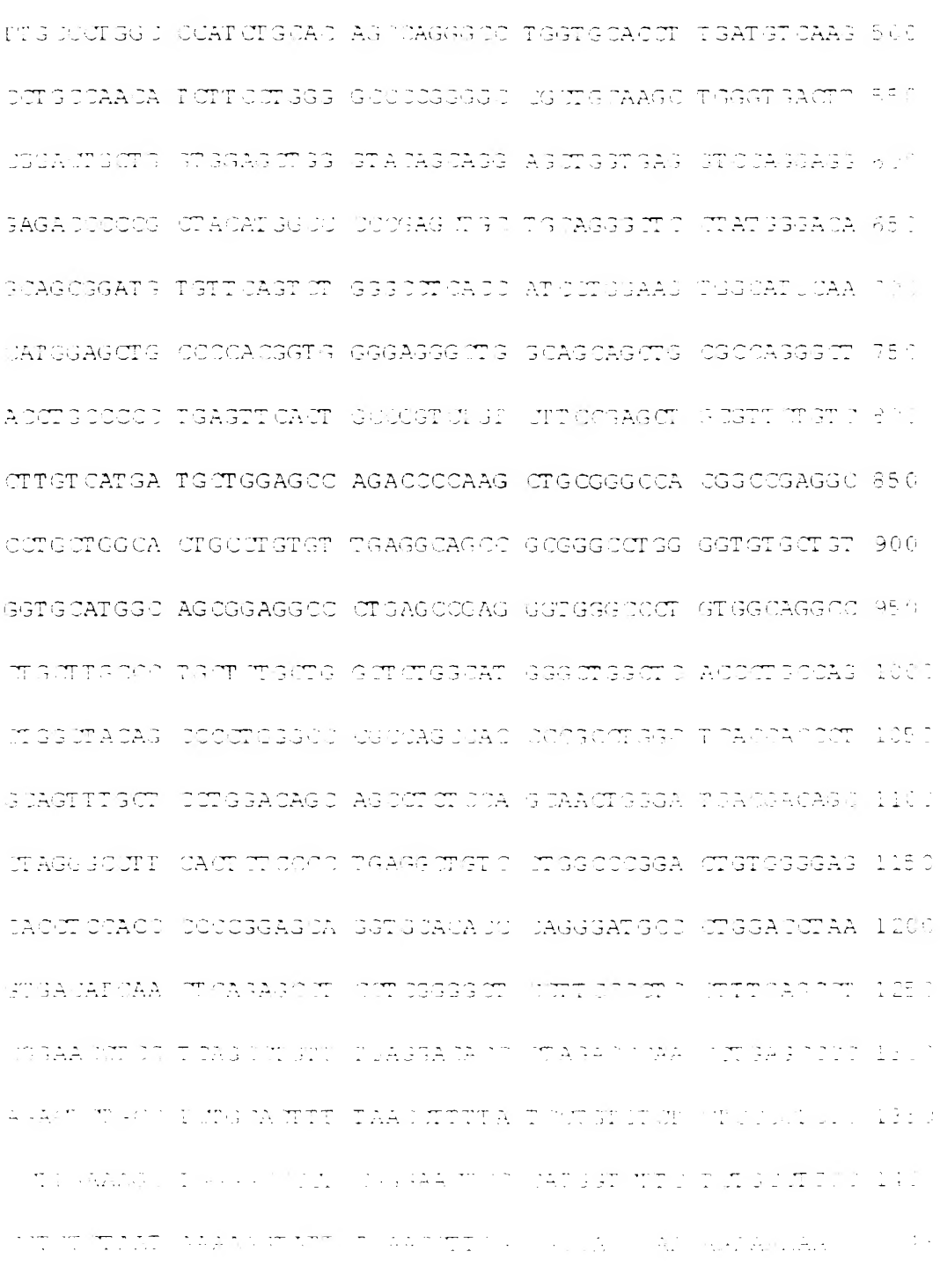

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(A) LENGTH: 479

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

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1				5					10					15
Arg	Gly	Glu	Ala	Ser	Glu	Thr	Leu	Gln	Ser	Pro	Gly	Tyr	Asp	Pro
				20					25					30
Pro	Arg	Pro	Gln	Ser	Phe	Phe	Gln	Gln	Ser	Phe	Gln	Arg	Leu	Ser
				35					40					45
Arg	Leu	Gly	His	Gly	Ser	Tyr	Gly	Glu	Val	Phe	Lys	Val	Asp	Ser
				50					55					60
Lys	Glu	Asp	Gly	Arg	Leu	Tyr	Ala	Val	Lys	Arg	Ser	Met	Ser	Pro
				65					70					75
Phe	Arg	Gly	Pro	Lys	Asp	Arg	Ala	Arg	Lys	Leu	Ala	Glu	Val	Gly
				80					85					90
Ser	His	Glu	Lys	Val	Gly	Gln	His	Pro	Cys	Cys	Val	Arg	Leu	Glu
				95					100					105
Gln	Ala	Trp	Glu	Glu	Gly	Gly	Ile	Leu	Tyr	Leu	Gln	Thr	Glu	Leu
				110					115					120
Cys	Gly	Pro	Ser	Leu	Gln	Gln	His	Cys	Glu	Ala	Trp	Gly	Ala	Ser
				125					130					135
Leu	Pro	Glu	Ala	Gln	Val	Trp	Gly	Tyr	Leu	Arg	Asp	Thr	Leu	Leu
				140					145					150
Ala	Leu	Ala	His	Leu	His	Ser	Gln	Gly	Leu	Val	His	Leu	Asp	Val
				155					160					165
Lys	Pro	Ala	Asn	Ile	Phe	Leu	Gly	Pro	Arg	Gly	Arg	Cys	Lys	Leu
				170					175					180
Gly	Asp	Phe	Gly	Leu	Leu	Val	Glu	Leu	Gly	Thr	Ala	Gly	Ala	Gly
				185					190					195
Glu	Val	Gln	Glu	Gly	Asp	Pro	Arg	Tyr	Met	Ala	Pro	Glu	Leu	Leu
				200					205					210
Gln	Gly	Ser	Tyr	Gly	Thr	Ala	Ala	Asp	Val	Phe	Ser	Leu	Gly	Leu
				215					220					225
Thr	Ile	Leu	Glu	Val	Ala	Cys	Asn	Met	Glu	Leu	Pro	His	Gly	Gly
				230					235					240
Glu	Gly	Trp	Gln	Gln	Leu	Arg	Gln	Gly	Tyr	Leu	Pro	Pro	Glu	Phe
				245					250					255
Thr	Ala	Gly	Leu	Ser	Ser	Glu	Leu	Arg	Ser	Val	Leu	Val	Met	Met
				260					265					270
Leu	Glu	Ile	Asp	Pro	Lys	Leu	Arg	Ala	Thr	Ala	Glu	Ala	Leu	Leu
				275					280					285

BNSDOCID <EP___0835937A2_1_>

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Cys Met Ala Ala Glu Ala Leu Ser Arg Gly Trp Ala Leu Trp Gln
305 310 315
Ala Leu Leu Ala Leu Leu Leu Trp Leu Trp His Gly Leu Ala His
320 325 330
Pro Ala Ser Trp Leu Gln Pro Leu Gly Pro Pro Ala Thr Pro Pro
335 340 345
Gly Ser Pro Pro Cys Ser Leu Leu Leu Asp Ser Leu Leu Ser Ser
350 355 360
Asn Trp Asp Asp Asp Ser Leu Gly Pro Ser Leu Ser Pro Glu Ala
365 370 375
Val Leu Ala Arg Thr Val Gly Ser Thr Ser Thr Pro Arg Ser Arg
380 385 390
Cys Thr Pro Arg Asp Ala Leu Asp Leu Ser Asp Ile Asn Ser Glu
395 400 405
Pro Pro Arg Gly Ser Phe Pro Ser Phe Glu Pro Arg Asn Leu Leu
410 415 420
Ser Leu Phe Glu Asp Thr Leu Asp Pro Thr Xaa Ala Pro Asp Ser
425 430 435
Ala Ser Ala Leu Leu Thr Phe Tyr Pro Val Ser Leu Pro Ser Pro
440 445 450
Leu Lys Ala Gly Ala Pro Arg Glu Leu Pro Trp Ser Ser Leu Pro
455 460 465
Gly Arg Val Xaa Xaa Lys Val Phe Glu Pro Trp Glu His Pro
470 475

(2) INFORMATION FOR SEQ ID NO: 3

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 508

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

BNSDOCID: <EP 0835937A2>

Asp Thr Phe Phe Lys Gln Cys Phe Lys Ser Ile Cys Leu Glu
Arg Gly Ser Phe Gly Glu Val Tyr Lys Val Gln Ser Leu Glu Asp
Gly Cys Phe Tyr Ala Val Lys Arg Ser Val Ser Pro Phe Arg Gly
Val Ser Asp Arg Gln Arg Lys Leu Gln Glu Val Arg Lys His Glu
Arg Val Gly Glu His Pro Asn Cys Leu Arg Phe Val Arg Ala Trp
Glu Glu Lys Arg Met Leu Tyr Leu Gln Thr Glu Leu Cys Ala Gly
Ser Leu Gln Gln His Ser Glu Glu Phe Ala Gly Ser Leu Pro Glu
Arg Arg Val Trp Asn Ile Thr Cys Asp Leu Leu His Gly Leu Lys
His Leu His Asp Arg Asn Leu Leu His Leu Asp Ile Lys Pro Ala
Asn Val Phe Ile Ser Phe Ser Gly Val Cys Lys Leu Gly Asp Phe
Gly Leu Met Val Glu Leu Asp Gly Thr Glu Gly Ser Gly Glu Ala
Gln Glu Gly Asp Pro Arg Tyr Met Ala Pro Glu Leu Leu Asp Gly
Ile Phe Ser Lys Ala Ala Asp Val Phe Ser Leu Gly Met Ser Leu
Leu Glu Val Ala Cys Asn Met Glu Leu Pro Lys Gly Gly Asp Gly
Trp Gln Gln Leu Arg Gln Gly His Leu Pro Thr Glu Phe Thr Ser
Asp Leu Pro Pro Asp Phe Leu Lys Val Leu Ser Ala Met Leu Glu
Pro Asp Pro Arg Arg Arg Ala Thr Val Asp Trp Leu Leu Asp Leu
Pro Ala Leu Arg Asn Ala Glu Arg Trp Arg Met Val Thr Leu Ala
Gln Glu Asp Thr Leu Gly Cys Leu Gly Ala Val Tyr Gln Phe
Val Trp Leu Leu Ser Phe Val Leu Trp Leu Asn Arg Pro Val
Leu Gly Thr Leu His Tyr Cys Arg Ala Leu Pro Arg
Pro Pro Cys Asn Cys Phe Pro Asp Gly Gln Ser Ser Pro
Ser Ser Asp Trp Asp Asp Asp Val Phe Glu

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385 390 395

Val Pro Pro Ser Pro Leu Ala Thr His Arg Asn Leu Thr Tyr His

400 405 410

Gly Gln Glu Leu Ile Gly Arg His Ser Pro Asp Leu Leu Ser Arg

415 420 425

Pro Ser Leu Gly Ser Thr Ser Thr Pro Arg Asn Leu Ser Pro Glu

430 435 440

Phe Ser Met Arg Lys Arg Ser Ala Leu Pro Leu Thr Pro Asn Val

445 450 455

Ser Arg Ile Ser Gln Asp Ser Thr Gly Lys Ser Arg Ser Pro Ser

460 465 470

Thr Ser His Ser Ser Ser Gly Phe Val Asp Ala Glu Val Gln Arg

475 480 485

Thr Leu Phe Leu Pro Arg Asn Leu Leu Gly Met Phe Asp Asp Ala

490 500 505

Thr Glu Gln

Claims

1. An isolated polynucleotide comprising a member selected from the group consisting of:

   (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising amino acids of SEQ ID NO: 2.
   (b) a polynucleotide which by virtue of the redundancy of the genetic code encodes the same amino acids of SEQ ID NO: 2.
   (c) a polynucleotide which is complementary to the polynucleotide of (a) or (b), and
   (d) a polynucleotide comprising at least 15 contiguous bases of the polynucleotide of (a), (b) or (c).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 2 comprising nucleotides set forth in SEQ ID NO: 1.

5. The polynucleotide of Claim 2 which encodes a polypeptide comprising amino acids of SEQ ID NO: 2.

6. A vector comprising the DNA of Claim 2.

7. A host cell comprising the vector of Claim 6.

8. A process for producing a polypeptide comprising expressing from the host cell of Claim 7 a polypeptide encoded by said DNA.

9. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of Claim 6 such that the cell expresses the polypeptide encoded by the human cDNA contained in the vector.

10. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid sequence of SEQ ID NO: 2.

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11. A polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 2

12. An agonist to the polypeptide of claim 10

13. An antibody against the polypeptide of claim 10

14. An antagonist to the polypeptide of claim 10

15. A method for the treatment of a patient having need of Myt-kinase comprising administering to the patient a therapeutically effective amount of the polypeptide of claim 10

16. The method of Claim 15 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*

17. A method for the treatment of a patient having need to inhibit Myt-1 kinase polypeptide comprising administering to the patient a therapeutically effective amount of the antagonist of Claim 14

18. A process for diagnosing a disease or a susceptibility to a disease related to expression of the polypeptide of claim 10 comprising determining a mutation in the nucleic acid sequence encoding said polypeptide

19. A diagnostic process comprising analyzing for the presence of the polypeptide of claim 10 in a sample derived from a host

20. A method for identifying agonists and antagonists of human Myt-1 kinase comprising

preparing a mixture containing Myt-1 kinase polypeptide of claim 10 and a substrate for Myt-1 kinase which undergoes phosphorylation
contacting the mixture with a test compound and
determining whether the test compound increases or decreases the kinase activity of Myt-1 by measuring phosphorylation of the substrate

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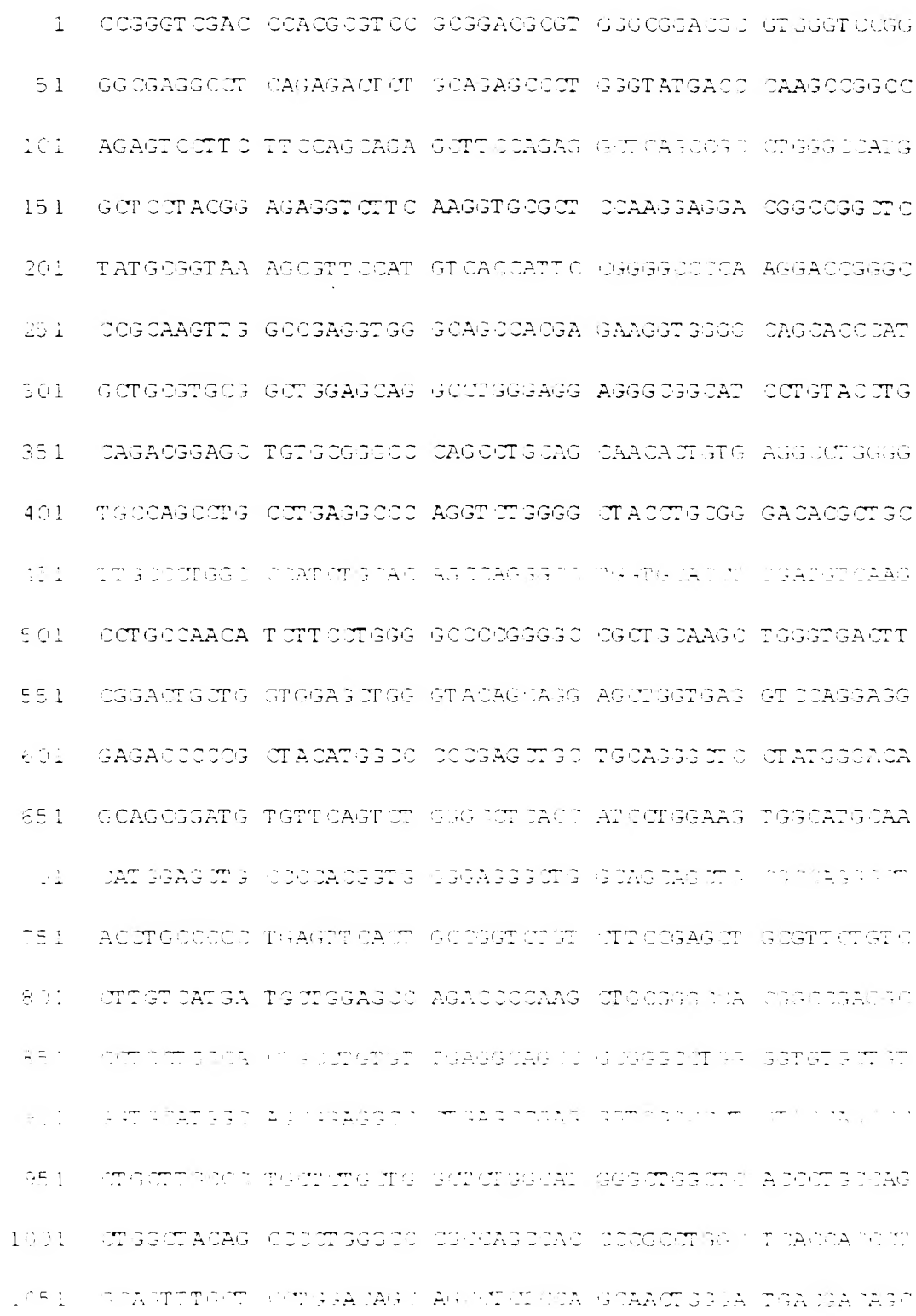
FIGURE 1: Partial Nucleotide Sequence of Human Myt-1 Kinase



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1151 CACCTCCACC CCCCGGAGCA GGTGCACACC CAGGGATGCC CTGGACCTAA

1201 GTGACATCAA CTCAGAGCCT CCTCGGGGCT CCTTCCCCTC CTTTGAGCCT

1251 CGGAACCTCC TCAGCCTGTT TGAGGACACC CTAGACCCAA CCTGAGCCCC

1301 AGACTCTGCC TCTGCACTTT TAACCTTTTA TCCTGTGTCT CTCCCGTCGC

1351 CCTTGAAAGC TGGGGCCCCT CGGGAACTCC CATGGTCTTC TCTGCCTGGC

1401 CGTGTCTAAT AAAAAGTATT TGAACCTTGG GAGCACCCAA AAAAAAAA

BNSDOCID <EP___0835937A2_I_>

FIGURE 2: Deduced Amino Acid Sequence of Human Myt-1 Kinase

```
   1  GSTHASADAW ADAWVRGEAS ETLQSPGYDP SRPESFFQQS FQRLSRLGHG
  51  SYGEVFKVRS KEDGRLYAVK RSMSPFRGPK DRARKLAEVG SHEKVGQHPC
 101  CVRLEQAWEE GGILYLQTEL CGPSLQQHCE AWGASLPEAQ VWGYLRDTLL
 151  ALAHLHSQGL VHLDVKPANI FLGPRGRCKL GDFGLLVELG TAGAGEVQEG
 201  DPRYMAPELL QGSYGTAADV FSLGLTILEV ACNMELPHGG EGWQQLRQGY
 251  LPPEFTAGLS SELRSVLVMM LEPDPKLRAT AEALLALPVL RQPRAWGVLW
 301  CMAAEALSPG WALWQALLAL LCWLWHGLAH PASWLQPLGP PATPPGSPPC
 351  SLLLDSSLSS NWDDDSLGPS LSPEAVLART VGSTSTPRSR CTPRDALDLS
 401  DINSEPPRGS FPSFEPRNLL SLFEDTLDPT *APDSASALL TFYPVSLPSP
 451  LKAGAPRELP WSSLPGRV** KVFEPWEHP
```


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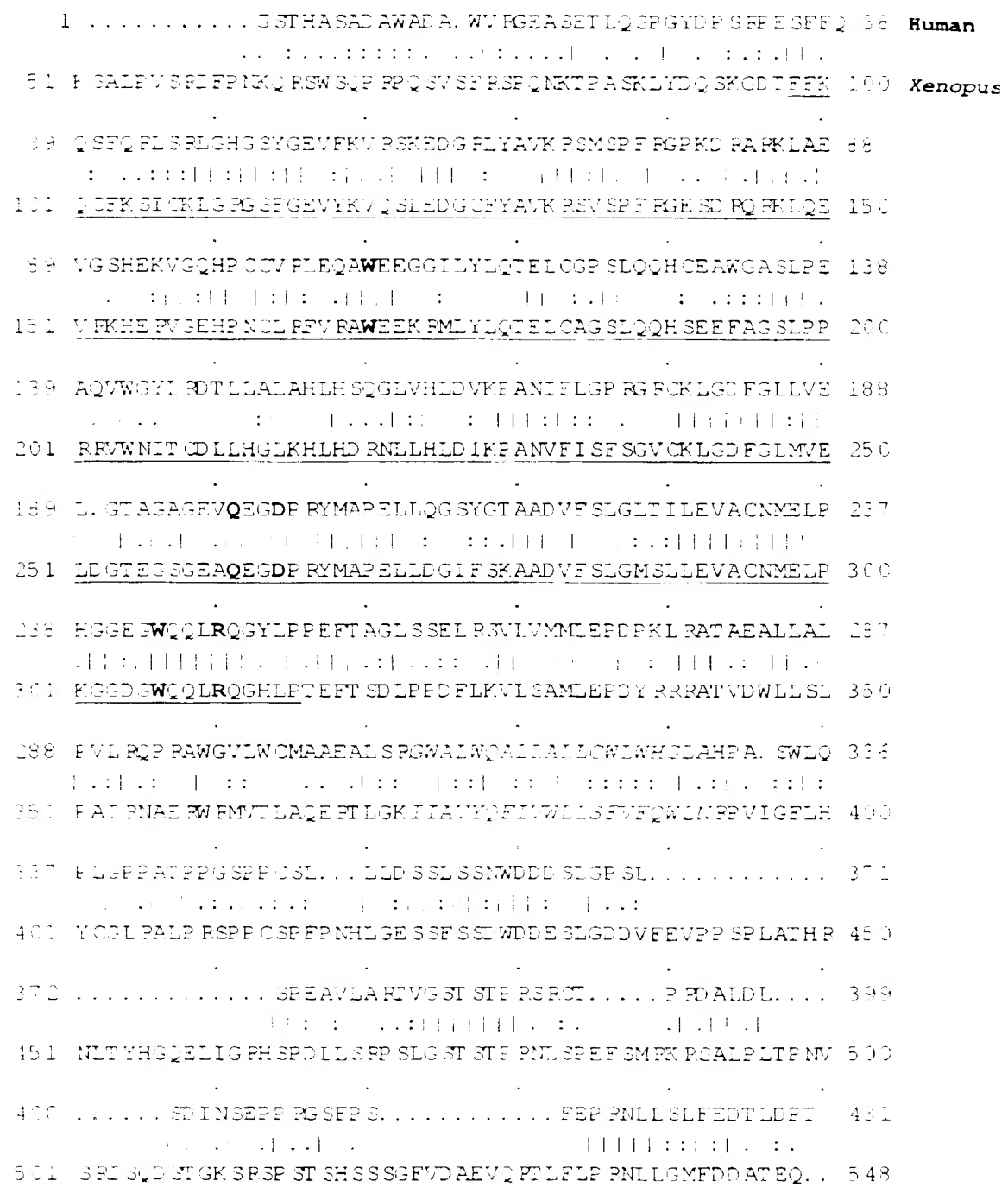
FIGURE 3: A comparison of the Deduced Amino Acid Sequence of Human Myt-1 vs *Xenopus* Myt-1



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(19) Europäisches Patentamt
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(12) EUROPEAN PATENT APPLICATION

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23.09.1998 Bulletin 1998/39

(43) Date of publication A2
15.04.1998 Bulletin 1998/16

(21) Application number **97308044.3**

(22) Date of filing **10.10.1997**

(51) Int Cl.[6] **C12N 15/54**, C12N 9/12, C12N 1/21, C07K 16/40, A61K 38/45

(84) Designated Contracting States
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority **11.10.1996 US 30389 P**

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(74) Representative **Crump, Julian Richard John et al**
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(54) Human MYT-1 kinase clone

(57) Human Myt-1 kinase polypeptides and DNA (RNA) encoding such enzyme and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such human Myt-1 kinase in the development of treatments for cancers such as leukemias, solid tumors and metastases, chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis, proliferative cardiovascular diseases such as restenosis, proliferative ocular disorders such as diabetic retinopathy and macular degeneration, and benign hyperproliferative diseases such as benign prostatic hypertrophy and hemangiomas, among others, are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides.

EP 0 835 937 A3

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 97 30 8044

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	DATABASE : EMBL SEQUENCES EMBL, Heidelberg, FRG Accession No. D61277, 27 August 1995 FUJIWARA T. ET AL.: "Human fetal brain cDNA GEN-174G10 (EST), similar to none." XP002057981 * abstract * ---	1-4,6-9	C12N15/54 C12N9/12 C12N1/21 C07K16/40 A61K38/45
P,X	LIU F. ET AL.: "The human Myt1 kinase preferentially phosphorylates Cdc2 on Thr14 and localizes to the endoplasmic reticulum and Golgi complex" MOL. CELL. BIOL., vol. 17, no. 2, February 1997, pages 571-583, XP002057978 * the whole document * ---	1-11	
A,D	ATHERTON-FESSLER S. ET AL.: "Cell cycle regulation of the p34/cdc2 inhibitory kinases" MOL. BIOL. CELL, vol. 5, no. 9, 1994, pages 989-1001, XP002057979 * abstract * --- -/--	1-20	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C12N
C07K
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or some or all of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for the following claims.

Claims searched completely:

Claims searched incompletely:

Claims not searched:

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	11 March 1998	Galli, I

CATEGORY OF CITED DOCUMENTS

X particularly relevant if taken alone
Y particularly relevant if combined with another document of the same category
A technological background
O non-written disclosure
P intermediate document

T theory or principle underlying the invention
E earlier patent document, but published on, or after the filing date
D document cited in the application
L document cited for other reasons

& member of the same patent family, corresponding document

BNSDOCID <EP___0835937A3_I_>

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 97 30 8044

Remark : Although claims 15-17 and 18 (the latter as far as methods in vivo are concerned) are directed to methods of treatment and diagnosis of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

BNSDOCID <EP 0835937A3_I_>

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 97 30 8044

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A,D	MUELLER P. ET AL.: "Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both Thr14 and Tyr15." SCIENCE, vol. 270, 6 October 1995, pages 86-90, XP002057980 * abstract * * figure 1 *	1-20	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1503 03.82 (P04C10)

BNSDOCID <EP___0835937A3_I_>